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PHARMACOKINETICS OF THE TRICHOTHECENE MYCOTOXIN, T-2 TOXIN, IN SWINE AND CATTLE

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V. R. BEASLEY, S. P. SWANSON, R. A. CORLEY, W. B. BUCK, G. D. KORITZ and H. R. BURMEISTER. Pharmacokinetics of the trichothecene mycotoxin, T-2 toxin, in swine and cattle. *Toxicon* 24, 13–23, 1986. — The pharmacokinetics of the trichothecene mycotoxin, T-2 toxin, were determined in growing gilts and heifers. Following intra-aortal administration in swine and intravenous administration in calves, the disappearance of the parent T-2 toxin followed a 2-compartment open model. Mean elimination phase half-lives were 13.8 and 17.4 min and mean apparent specific volumes of distribution were 0.366 and 0.376 l/kg in swine and calves, respectively. The fraction of T-2 toxin eliminated as parent compound in the urine was negligible. In spite of administration of a lethal oral dose in swine (2.4 mg/kg) and toxic oral doses (up to 3.6 mg/kg) in calves, no parent T-2 toxin was detected in plasma or urine.

After intra-aortal administration in swine, tissue concentrations of T-2 toxin were consistently highest in lymphoid organs. Tissue residues of T-2 toxin were rapidly depleted such that, in spite of administration of a potentially lethal intra-aortal dose, no quantifiable T-2 toxin was present in any of the tissues collected at 4 hr after dosing. No T-2 toxin could be detected in liver, even at 1 hr after dosing.

INTRODUCTION

T-2 TOXIN is a 12,13-epoxytrichothecene (trichothecene) mycotoxin. Outbreaks of mycotoxicoses in farm animals have been associated with T-2 toxin in feeds in the United States (HSU *et al.*, 1972), Canada (PULS and GREENWAY, 1976), Japan (UENO, 1977) and the Soviet Union (FORGACS and CARLL, 1962; JOFFE and YAGEN, 1977). T-2 toxin is produced especially by *Fusaria*; isolates of *F. sporotrichioides* which produced T-2 toxin were consistently isolated from grains associated with alimentary toxic aleukia in humans (JOFFE and YAGEN, 1977). More recently, T-2 toxin was detected on leaves, in water and in a yellow powder in association with alleged 'Yellow Rain' chemical warfare attacks in Asia (ROSEN and ROSEN, 1983; MIROCHA *et al.*, 1983).

With regard to physical and metabolic alterations of T-2 toxin, it was previously found that T-2 toxin was not altered in significant amounts by serum, blood (OHTA *et al.*, 1977) or simulated gastric juice (ELLISON and KOTSONIS, 1974). Moreover, when *F. tricinctum* cultures were extracted with water, artificial digestive tract secretions or rat bile for subsequent LD_{50} studies in rats, all extracts had similar LD_{50} values (KOSURI *et al.*, 1971). When a ruminal fluid extract of the culture was tested, however, the LD_{50} was almost 2 times as high as any other extract.

The administration of radiolabelled T-2 toxin has generally revealed initial concentration of radioactivity in the liver and kidneys, followed by rapid biliary and urinary excretion (CHI *et al.*, 1978; MATSUMOTO *et al.*, 1978). At 18 hr after swine were given radiolabelled T-2 toxin by intubation, the voided feces and urine accounted for 25% and 20% of the radioactivity, respectively (ROBISON *et al.*, 1979). The concentration of radiolabel in bile was roughly 30 times that in kidney and liver, the tissues with the highest concentrations. In contrast, spleen contained only 1/3 of the renal concentration. Fifty per cent of the radioactivity was assumed to be in the gastrointestinal tract.

After tritiated T-2 toxin was administered orally to a Jersey cow at 0.42 mg/kg, approximately 70% and 30% of the administered radiolabel appeared in the feces and urine, respectively, by 72 hr (YOSHIZAWA *et al.*, 1981). The authors indicated that at 4 hr after dosing, unmetabolized T-2 toxin was present in plasma at 8 ppb. T-2 toxin and known metabolite products of esterase cleavage, including HT-2 toxin, neosolaniol and 4-deacetylneosolaniol, accounted for only minor amounts of the radiolabel in urine, milk and plasma. Subsequently, 3′-hydroxy T-2 toxin and 3′-hydroxy HT-2 toxin were identified as more significant metabolites in plasma, urine and milk of this cow, but the time course of the occurrence of these metabolites has not yet been thoroughly evaluated (YOSHIZAWA *et al.*, 1982).

The purpose of this study was to delineate the rate of disappearance of T-2 toxin from plasma and urine and to determine the likelihood of residues of T-2 toxin in the edible tissues of swine and cattle.

MATERIALS AND METHODS

Studies in swine

Fourteen female swine, 26 – 66 kg (mean 49 ± 12; $\bar{x}$ ± S.D.), of mixed breeding were used. The gilts were immunized against erysipelas (Rhusigen, Pitman-Moore, Inc., Washington Crossing, NJ) acclimatized for at least 1 week and allotted to 1 of 6 groups. After administration of atropine, anesthesia was induced and maintained by inhalation of halothane and oxygen. An indwelling catheter was surgically implanted in the aorta via the femoral artery. Postoperatively, a foley catheter was inserted into the urinary bladder and the animal was housed in a metabolism cage. The pigs were dosed with T-2 toxin no sooner than 2 days postoperatively. All animals were normal in appearance and behavior at the time of dosing.

The T-2 toxin was solubilized in 2.5 ml of 50% ethanol and was administered in single intra-aortal (i.a.) doses to 2 gilts in each of groups 1 – 4 at the following doses: 0 (vehicle control), 0.3, 0.6 and 1.2 mg/kg. Four gilts in group 5 received T-2 toxin at a dose of 1.2 mg/kg i.a. and were killed at 1 – 4 hr after dosing. Two swine in group 6 were dosed with T-2 toxin intragastrically at 2.4 mg/kg. The dose was solubilized in 2.5 ml of 50% ethanol and the tube thoroughly flushed with water. Swine were necropsied immediately after death or euthanasia. Blood samples were collected in heparinized syringes prior to dosing and at the following postinjection times (min): 2.5, 5.0, 7.5, 10, 15, 20, 25, 30, 40, 45, 50, 55, 60, 70, 80, 90, 110, 120, 150 and 180. The blood samples were chilled in ice water and centrifuged. The plasma was collected and frozen until assayed. Urine volumes were measured and samples were collected prior to dosing and at 30 min intervals thereafter. Urine samples were kept on ice during collection and then frozen until assayed. Feces and vomitus were collected and put on ice as they became available and subsequently frozen prior to analysis. Spleen, mesenteric lymph nodes, muscle, kidney, liver, bile and the contents of the stomach, jejunum, ileum and colon were routinely collected at necropsy and flash-frozen prior to analysis.

Studies in calves

Seven female calves weighing 201 – 268 kg (mean 238 ± 26 S.D.) were used in the study after being dewormed with thiabendazole, vaccinated against infectious bovine rhinotracheitis and parainfluenza-3 (Nasalgen IP, Jensen-Salisbury Laboratories, Division of Burroughs Wellcome Co., Kansas City, MO) and dosed with *Leptospira* (Leptomune 5, Beecham Laboratories, Bristol, TN) and *Clostridia* (Clostroid CSN, Fort Dodge Laboratories, Fort Dodge, IA) bacterins. After a 3 week or longer acclimation period, the calves were placed in metabolism cages on the day prior to dosing. Bilateral jugular cannulas and foley urinary catheters were inserted on the day of dosing.

In preliminary studies, calf C1 was given T-2 toxin i.v. at 0.15 mg/kg and orally at 0.6 and 1.2 mg/kg. Dosings in this calf were at least 2 weeks apart. T-2 toxin in 5 ml of 70% ethanol was administered to two calves

each at 0.6 and 1.2 mg/kg i.v. The orally dosed calves were given crystalline T-2 toxin in dextrose via gelatin capsules. One calf, C2, was given T-2 toxin at 2.4 mg/kg in 2 oral doses spaced 3 weeks apart and was killed at 24 hr after the second dose. Another calf, C3, was given a single oral dose of 3.6 mg/kg. Plasma and urine were collected at the same time points as in swine. Feces were collected as voided.

One calf, C5, which died 10.5 hr after being given T-2 toxin at 1.2 mg/kg, was necropsied immediately after death. Another calf, C7, was given T-2 toxin at 0.6 mg/kg i.v. and was killed at 6½ hr. The vehicle control calf C8, as well as C4 dosed at 0.6 mg/kg i.v., were killed at approximately 24 hr. Calves were killed by i.v. injection of pentobarbital followed by exsanguination.

Samples collected from calves at necropsy for T-2 toxin analysis included spleen, mesenteric lymph nodes, muscle, kidney, liver, bile and the contents of the rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, spiral colon and large colon. In addition, the brain and heart of calf no. C5 were analyzed. These samples were flash-frozen prior to analysis.

Analytical methods

Plasma and urine. Iso T-2, an isomer of T-2 toxin, was added to samples as an internal standard prior to extraction with benzene. The extracts were partitioned against aqueous sodium hydroxide, chromatographed on a small florisil column and eluted with chloroform – methanol (95:5 v/v). After concentration of the eluate, the heptafluorobutyryl derivative of T-2 toxin was prepared by reaction with heptafluorobutyrylimidazole (SWANSON *et al.*, 1983). The T-2 and iso T-2 derivatives were separated by gas-liquid chromatography at 230°C using a 1.8 m column containing 3% OV-1 and quantitated with a nickel 63 electron capture detector. Recoveries averaged 98 ± 5% ($\bar{x}$ ± S.D.; n = 29) at concentrations ranging from 50 to 1000 ng/ml.

Tissues. After addition of the internal standard (iso T-2), the minced tissues were extracted by blending with acetone. Lead acetate was added to the filtrate to precipitate proteins and pigments. The extract was then defatted with hexane. T-2 toxin was partitioned into chloroform and the chloroform layer was subsequently partitioned against aqueous sodium hydroxide. The chloroform layer was added to a 2.5 g florisil column and the column rinsed with dichloromethane followed by chloroform – acetone (97:3 v/v). T-2 toxin was eluted with chloroform – methanol (95:5 v/v). After concentration of the eluate, the residue was redissolved in 0.5 ml toluene. Derivatization and quantitation was then performed as described for plasma and urine. Recoveries of T-2 toxin in tissues averaged 105 ± 20% ($\bar{x}$ ± S.D.; n = 18).

Bile. Bile was analyzed by adaptation of a technique developed to quantitate diacetoxyscirpenol in plasma and urine (SWANSON *et al.*, 1982). Bile, to which the internal standard iso T-2 was added, was placed on a 500 mg C-18 Bond Elute cartridge (Analytichem International, Harbor City, CA). The cartridge was rinsed with water followed by methanol – water (3:7 v/v). T-2 toxin was subsequently eluted with methanol – water (9:1 v/v). Sodium chloride solution was added to the eluate and the mixture was partitioned 3 times with toluene – ethyl acetate (9:1 v/v). Hexane was added to the combined organic layers and transferred to a 1 g florisil column. Thereafter, the method was identical to that used for plasma and urine. Recoveries of T-2 toxin in bile averaged 78 ± 12% ($\bar{x}$ ± S.D.; n = 6).

Feces, vomitus, gastrointestinal tract contents. Except for mincing, these specimens were processed in the same manner as tissues. The recovery of T-2 toxin in feces was 77 ± 10% ($\bar{x}$ ± S.D.; n = 8). In tissues, body fluids and gastrointestinal contents, the limits of detection and reliable quantitation for T-2 toxin were 25 and 40 ng/ml, respectively.

Data analysis. Following intravascular administration of T-2 toxin to swine and calves, visual inspection of semilogarithmic plots of plasma concentrations vs. time suggested a biexponential equation of the form $Cp = Ae^{-\alpha t} + Be^{-\beta t}$, where Cp is the plasma concentration at a given time t, A and B are coefficients and α and β are exponents. Best fit values were determined using a digital computer (IBM 360/75) and an iterative least squares fitting program, the SAAM-27 program (BERMAN and WEISS, 1968). The parameters of the biexponential equation were then solved for the first order rate constants of a two compartment open model. Inspection of the plasma data of P17 and C5 revealed that, at the high dose, these were the individuals of each species with the slowest elimination phases as well as the most pronounced transition between α and β phases. To determine whether a more complex model would better explain the disappearance of T-2 toxin from plasma, the data of P17 and C5 were fitted to a 3 compartment open model. An F test (BOXENBAUM *et al.*, 1974) of the weighted (by 1/observation squared) sums of squared deviations of the data points from the predicted points of the two and three compartment models was used to determine whether significant improvement of fit had resulted from the use of the more complex model.

The terminal portion of the plasma curve of P21 was skewed upward due to comparatively similar values of 53, 55 and 48 ng/ml at 55, 60 and 70 min, respectively. The plasma T-2 concentration at the next time point, 80 min, had declined to the limit of detection. It was apparent, therefore, that the least squares computation had entered a local minimum in the fitting of estimates of the parameter values (BOXENBAUM *et al.*, 1974), which caused inappropriate estimation of the terminal slope. Therefore, in order to obtain best fit estimates of the kinetic parameters for P21, the program was run with β fixed at the mean β value of the other 5 animals at this dose.

In order to assess whether dosage had influenced the pharmacokinetics of T-2 toxin, the best fit values of kinetic parameters derived in calves at each of the two doses were compared by t-tests (STEEL and TORRIE, 1960).

Similarly, all kinetic parameters obtained over the three doses in swine were compared by a one-way analysis of variance, as well as by linear and curvilinear regression using a computer program (Statistical Package for the Social Sciences, SPSS, Inc., Chicago, IL).

RESULTS

Swine of this study given the oral or the high i.a. dose and one of the calves given the high i.v. dose experienced circulatory shock and died. Shock began to be apparent within the first few hours and deaths occurred between 10.5 and 20 hr after dosing. A more detailed description of these effects and tissue changes in these animals will be reported in a subsequent paper.

Individual and mean pharmacokinetic parameters determined for intravascularly dosed swine and cattle are shown in Tables 1 and 2. Figures 1 and 2 are curves calculated by the mean kinetic parameters at each dosage to illustrate the agreement with the mean T-2 concentration at each time point. In both P17 and C5, no significant improvement in the

TABLE 1. ESTIMATES OF TOXICOKINETIC PARAMETERS IN SWINE AFTER DOSING INTRA-AORTALLY WITH T-2 TOXIN*

Animal number	Dose (mg/kg)	α (per min)	β (per min)	A (ng/ml)	B (ng/ml)	$T'CL$ (ml/kg/min)
P4	0.3	0.371	0.058	613	92	93
P9	0.3	0.500	0.061	283	165	92
$\bar{x}$		0.436	0.060	448	129	92
± S.D.		±0.091	±0.002	±233	±52	±1
P7	0.6	0.576	0.046	2748	166	72
P12	0.6	0.579	0.101	1608	349	96
$\bar{x}$		0.578	0.074	2178	258	84
± S.D.		±0.002	±0.039	±806	±129	±17
P8	1.2	0.488	0.063	4018	1071	48
P13	1.2	0.344	0.069	3876	1676	34
P15	1.2	0.242	0.033	3388	268	54
P17	1.2	0.240	0.026	1855	204	77
P21	1.2	0.163	0.052	1957	895	41
P26	1.2	0.478	0.067	4248	315	88
$\bar{x}$		0.326	0.052	3324	738	57
± S.D.		±0.135	±0.018	±1059	±583	±21

*α and β were derived from the slopes of the distributive and elimination phases which are $-\alpha/2.303$ and $-\beta/2.303$, respectively. A and B are the respective intercepts of the distributive and elimination phases. $T'Cl$ is the theoretical rate of total body clearance and is calculated from $\beta \times Vd$ area.

TABLE 2. ESTIMATES OF TOXICOKINETIC PARAMETERS IN CALVES AFTER I.V. DOSING WITH T-2 TOXIN*

Animal number	Dose (mg/kg)	α (per min)	β (per min)	A (ng/ml)	B (ng/ml)	$T'CL$ (ml/kg/min)
C4	0.6	0.202	0.033	1043	139	64
C7	0.6	0.208	0.047	1146	295	51
$\bar{x}$		0.205	0.040	1095	217	58
± S.D.		±0.004	±0.010	±73	±110	±9
C5	1.2	0.442	0.039	4020	309	70
C9	1.2	0.252	0.043	3199	789	39
$\bar{x}$		0.347	0.041	3610	549	55
± S.D.		±0.134	±0.003	±581	±339	±22

*For definitions of terms see Table 1.

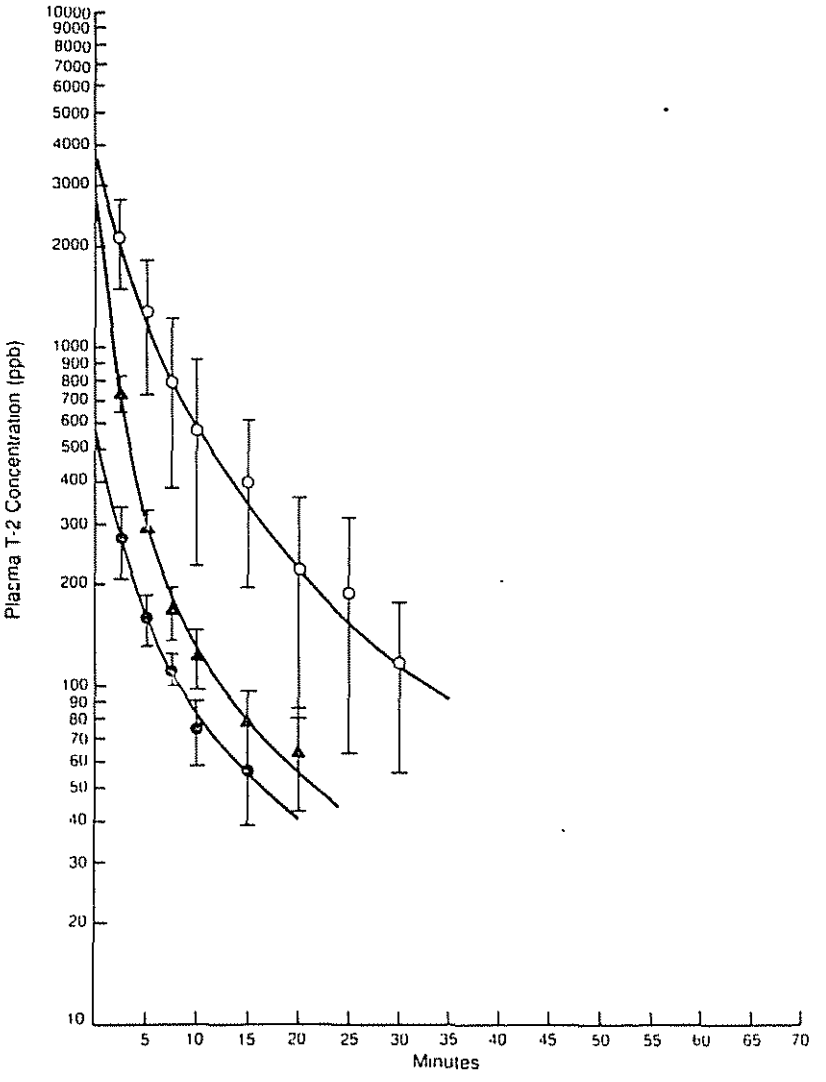

FIG. 1. SWINE PLASMA T-2 CONCENTRATIONS AFTER 1.2 (○), 0.6 (▲) or 0.3 (●) mg/kg I.A. Results are shown as mean ± S.D. (n = 2 at the two lower doses; n = 6 at the high dose).

sums of squares resulted from the use of the 3 compartment model, as compared to the two compartment model.

One-way analysis of variance of the effect of different i.a. dosages of T-2 toxin on the pharmacokinetic parameters in swine did not reveal any significant differences between the three groups. The calculation of linear regression coefficients similarly revealed no linear trend in any of the parameters. When analyzed by multiple regression, a significant curvilinear trend was apparent in only one parameter, total body clearance, which declined at an increasing rate as dosage increased (Fig. 3). In calves, t-tests of the parameters revealed no differences between the two intravascular dosages. Also in calves, the mean total body clearances in the 0.6 and 1.2 mg/kg i.v. groups were essentially the same.

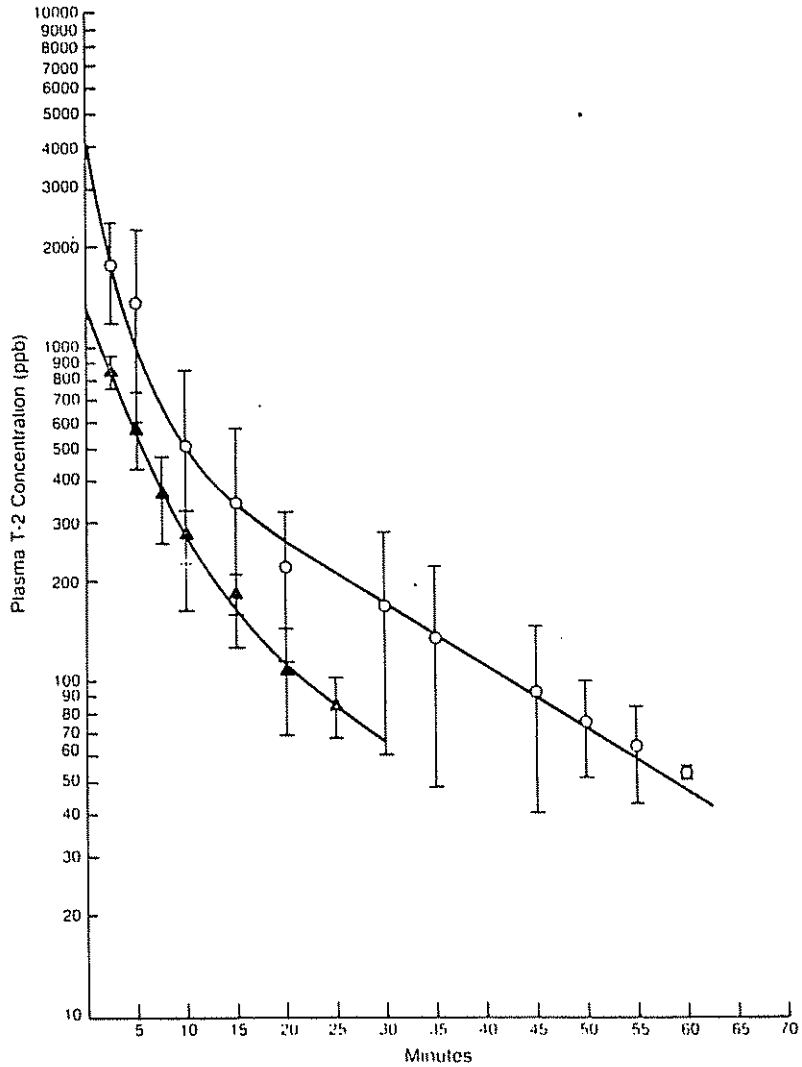

FIG. 2. BOVINE PLASMA T-2 CONCENTRATIONS AFTER 1.2 (○) OR 0.6 (▲) mg/kg I.V. Results are shown as mean ± S.D. (n = 2).

T-2 toxin rapidly disappeared from the plasma of intravascularly dosed swine and cattle. By 2 hr after intravascular administration of T-2 toxin at 1.2 mg/kg, the toxin could no longer be detected in the plasma of either species. Although severe cardiovascular shock and renal shutdown occurred in individuals of both species at this 1.2 mg/kg dose, urine production continued at lower doses. Regardless of which species and which intravascular dosage of T-2 toxin was employed, urinary excretion of T-2 toxin as the parent compound was inconsistent, and less than 0.1% of the dose was recovered in urine as parent compound.

Analysis of tissues of swine dosed with T-2 toxin at 1.2 mg/kg i.a. and killed after 1 – 4 hr revealed rapid disappearance of the parent compound (Fig. 4). The lymphoid organs, spleen and mesenteric lymph nodes, contained the highest concentration of parent T-2 toxin. Muscle tissue contained more of the parent compound than kidney, in which

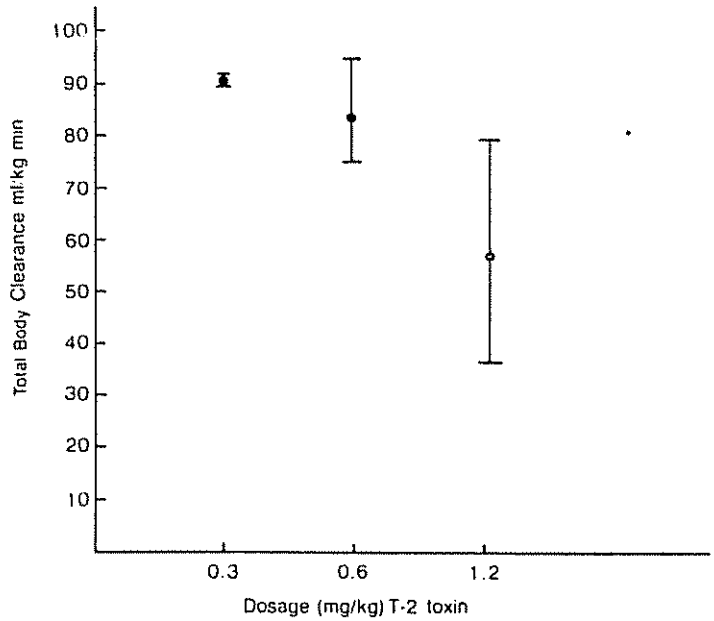

FIG. 3. TOTAL BODY CLEARANCE OF T-2 TOXIN IN SWINE (MEAN ± S.D.) AS A FUNCTION OF I.A. DOSAGE. $n = 2$ for each of the two lower doses; $n = 6$ at the high dose).

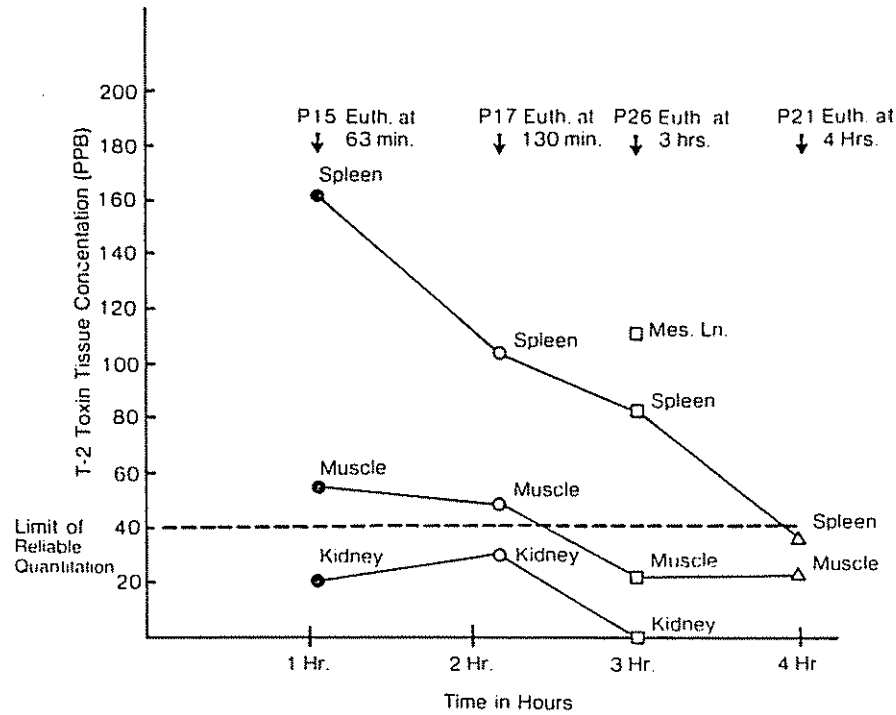

FIG. 4. SWINE T-2 TOXIN CONCENTRATIONS IN TISSUES WHEN ANIMALS WERE KILLED FROM 1 TO 4 hr AFTER I.A. DOSES OF 1.2 mg/kg.
T-2 toxin was never present at detectable concentrations in the liver. Times shown at top of figure were times that animals were killed (Euth.) ($n = 1$).

T-2 concentrations were below the limit of reliable quantitation even at 1 hr. In liver and adipose tissue, no T-2 toxin was detected at any time. T-2 toxin was detected in the bile of only one animal and was present only at a concentration below the 40 ppb limit of reliable quantitation. This pig, P17, had received T-2 toxin at 1.2 mg/kg i.a. and was killed at 130 min. Calves and other swine, including P15 which was killed at 1 hour after an i.a. dose of 1.2 mg/kg, did not have detectable amounts of T-2 toxin in bile.

After P17, P21 and P26 received T-2 toxin at 1.2 mg/kg i.a., no parent compound was detected in 15 specimens of vomitus collected from 15 to 120 min after dosing. However, after intragastric administration of T-2 toxin at 2.4 mg/kg, vomiting occurred between 63 and 285 min postdosing in P14 and from 33 to 295 min in P15. Intact T-2 toxin in the vomitus accounted for approximately 12% and 36% of the dose and the final specimens of vomitus contained 15 and 35 ppm in P14 and P16, respectively.

Swine nos. P15, P26 and P13 were dosed at 1.2 mg/kg i.a. and were necropsied at 1.1, 3 and 12.3 hr after dosing, respectively. T-2 toxin was present, but not above the quantifiable limit of 40 ppb, in the stomach contents of P15. However, no T-2 toxin was detected in jejunum, cecum and colon contents of this animal nor was the parent compound detected in any of the gastrointestinal tract contents of P26 and P13. Similarly, feces from swine dosed with T-2 toxin at 0.6 or 1.2 mg/kg i.a. and voided at 2, 20, 25, 30, 118, 202, 209, 210 and 345 min postdosing contained no detectable T-2 toxin.

No T-2 toxin was detected in 4 specimens of feces voided by P14 from 3.2 to 8.5 hr after intragastric dosing at 2.4 mg/kg. However, the stomach, jejunal and spiral colon contents of this pig, which died at 19.5 hr after dosing, contained 16.4 ppm, 374 ppb and 66 ppb of T-2 toxin, respectively. The ileum and cecum of this animal were essentially empty. These results were somewhat different to those of P16, which died 18 hr after the same intragastric dose. The stomach contents of P16 contained 3.3 ppm, while the contents of the ileum, jejunum, cecum and colon were all free of detectable concentrations of T-2 toxin.

No T-2 toxin was detected in any tissues from cattle dosed i.v. or orally with T-2 toxin. These included: C2 dosed with 2.4 mg/kg orally and killed at 24 hr; C7 dosed with 0.6 mg/kg i.v. and killed at 5.5 hr; C5 dosed at 1.2 mg/kg i.v. and which died at 10.5 hr; calves killed at approximately 24 hr after an i.v. dose of 0.6 or 1.2 mg/kg. After C3 was dosed orally with T-2 toxin at 3.6 mg/kg, no intact T-2 toxin was detected in feces collected at 90, 170, 195, 585, 785, 1875, 2910, 3510 and 4260 min after dosing. At 24 hr after an oral dose of 2.4 mg/kg, concentrations of T-2 toxin which were detected in the gastrointestinal tract of C2 included 62 and 40 ppb in rumen and omasum contents, respectively. No intact T-2 toxin was detected in contents of the reticulum, abomasum, jejunum, cecum or spiral colon, or in the feces of this animal.

DISCUSSION

It was not concluded that the pharmacokinetics of T-2 toxin in swine and calves were independent of dosage for two reasons: the number of animals at most dosages was small, and there was a decrease in total body clearance with increasing dosage in swine. It is possible that circulatory or biochemical alterations induced by T-2 toxin may have impaired total body clearance at the higher doses. However, since the range of values for mean total body clearance values in swine was from 57 to 92 ml/kg/min, the differences among these dosages were small. Because of the rapid disappearance of the parent compound, the terminal plasma concentration curve was less clearly defined at the lower

dosages and this could have led to over-estimation of the total body clearance. In P4 and P9, plasma concentrations fell below the limit of detection at 20 and 25 min, respectively.

In swine, the fractions of total cardiac output distributed to the liver (via the hepatic artery) and to the gastrointestinal tract were reported to be 4.27% and 18.71%, respectively (TRANQUILLI *et al.*, 1982). These values, based on a cardiac index value of 135 ml/min/kg (TRANQUILLI *et al.*, 1982), are equivalent to approximately 5.8 and 25.7 ml/kg/min or an approximate sum of 31.5 ml/kg/min. Therefore, even if one were to assume that (1) the gastrointestinal and hepatic blood flow was normal during T-2 toxicosis, (2) 100% of the gastrointestinal blood flow was delivered via the portal vein to the liver, (3) 100% of the T-2 toxin in plasma in the portal vein and hepatic artery was cleared, and (4) an equilibrium occurred such that plasma concentrations of T-2 toxin remained proportional to the overall concentration of parent compound in the body as a whole, the amount delivered to the liver would still be insufficient to account for the clearance of T-2 toxin in this species. It is possible, therefore, that hepatic blood flow was altered and/or that metabolism in tissues other than the liver and gastrointestinal tract also contributed to the rapid disappearance of T-2 toxin from plasma. Nevertheless, the failure to detect T-2 toxin in liver, even at 1 hr after i.a. dosing, is compatible with evidence that this organ is a primary site of T-2 toxin modification. Moreover, the failure to detect T-2 toxin in plasma or urine at any time after intragastric or oral administration indicates a very active first-pass effect.

In a previous study in which a cow was orally dosed with unlabelled T-2 toxin daily for 2 days followed by radiolabeled T-2 toxin the third day (0.4 – 0.5 mg/kg each dose), 30% of the radiolabel was recovered in the urine (YOSHIZAWA *et al.*, 1981). That study and the present study indicated that only minimal amounts of T-2 toxin are eliminated in urine as the parent compound. The previous report cited the presence of 44, 11 and 3 ppb of actual T-2 toxin in the urine at 12, 24 and 48 hr, respectively. In the present study, however, no parent T-2 toxin was detected in multiple urine samples collected at regular intervals from 30 min to 27 hr after oral dosing of calves at 0.6, 1.2, 2.4 or 3.6 mg/kg. Whether these differences were due to the effects of diet, age, breed, multiple vs. single doses, decomposition of conjugated T-2 toxin or other differences is unknown.

T-2 toxin is apparently relatively poorly absorbed from and stable in the porcine stomach (the latter finding is in agreement with the *in vitro* studies of ELLISON and KOTSONIS, 1974). Swine given T-2 toxin intragastrically experienced shock terminating in death within 20 hr, and alterations in circulation from shock and the local effects of T-2 toxin may have affected functions of the gastrointestinal tract. Nevertheless, after T-2 toxin entered the small intestine, absorption and/or metabolic alteration rapidly occurred. Although radiolabel from T-2 toxin is eliminated via the bile (ROBISON *et al.*, 1979; CHI *et al.*, 1978; MATSUMOTO *et al.*, 1978), intact, unconjugated T-2 toxin was not present in significant amounts at necropsy in the bile of the calves or swine of the present study.

Calves administered T-2 toxin orally at up to 3.6 mg/kg survived the shock syndrome and were recovering at 24 hr. Ruminal dilution would undoubtedly delay absorption. Moreover, binding to or inactivation by components of the rumen fluid may occur, in agreement with evidence suggesting that rumen fluid may lessen the toxicity of *Fusarium* extracts (KOSURI *et al.*, 1971). Swine were given T-2 toxin in an ethanol – water (50:50 v/v) solution which was flushed from the stomach tube with water, while calves received 100% of the dose in crystalline form. Thus, in addition to obvious differences between the rumen contents of calves and the stomach contents of swine, dissolution of T-2 toxin must

also be considered. Nevertheless, in view of the pronounced clinical signs of T-2 toxicosis in C2 and C3, the failure to recover T-2 toxin in the feces of these calves, and the minimal amounts of T-2 toxin in the gastrointestinal tract contents of C2, dissolution and absorption undoubtedly occurred over the course of the experiment.

After i.a. administration in swine, lymphoid tissues consistently had the highest intact T-2 toxin concentrations. Although lymphocytes are most sensitive to T-2 toxin, it could not be concluded that the high concentrations were a result of binding to these cells. Previous studies with T-2 toxin in swine revealed much higher radiolabel concentrations in liver and kidney than in spleen (ROBISON *et al.*, 1979). It is possible that cytotoxic effects in the T-2 sensitive lymphocytes and a secondary reduction in blood supply to damaged lymphoid tissues may have increased the persistence of the parent toxin.

It is concluded that significant residues of T-2 toxin as parent compound are unlikely to be encountered in the edible tissues, plasma, urine or feces of naturally exposed swine and cattle. Thus, with regard to tissues, body fluids and feces, efforts to identify and characterize the time course of the principal metabolites of T-2 toxin may be of greater diagnostic value than analysis for the parent compound. When feedstuffs are unavailable but suspect feed was being consumed antemortem, it would be reasonable to analyze stomach or rumen contents for T-2 toxin. Diagnosticians should be aware of the possible dermal, oral, gastrointestinal, reproductive and immunosuppressive effects of the more toxic trichothecene mycotoxins, in addition to their preponderance in causing feed refusal.

Work in progress in our laboratories has revealed that 3′-hydroxy HT-2 toxin and glucuronide conjugates of 3′-hydroxy HT-2, 3′-hydroxy T-2, HT-2 and T-2 are the primary metabolites in swine. The time course of occurrence of these compounds will be characterized in subsequent reports.

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